THE APPLICATION OF CERTAIN LAWS OF PHYSICAL CHEMISTRY IN THE STANDARDIZATION OF DISINFECTANTS.*

EARLE B. PHELPS.

(From the Biological Laboratories of the Massachusetts Institute of Technology.)

The idea of standardizing disinfectants was first seriously proposed by Rideal and Walker.[1] Previous to that time, despite the fact that the germicidal properties of a great many chemical substances had been thoroughly investigated, no scientific attempt had ever been made to establish a common basis of comparison. The results of any disinfection experiments are fundamentally influenced by such conditions as temperature, character of the organism employed, number of organisms in unit volume, and character of the medium. In the absence of complete data covering these points results are practically worthless, at least for purposes of comparison. Even with such data given it is still impossible, owing to the variable conditions obtaining in practice, to establish any relationship, or order of excellence, among the various disinfectants. At best we can only hope to establish such relationship under specified experimental conditions. Rideal and Walker proposed to establish phenol as a standard. This selection was made because phenol is a substance readily obtainable anywhere in a comparatively pure condition. The effect of phenol and that of any other disinfectant may be determined simultaneously using the same test organism. The germicidal power of the disinfectant under investigation is then expressed as the "carbolic-acid coefficient," this being the ratio of the concentrations of the two disinfectants which will kill all the test organisms in the same time. Rideal and Walker recognized also that the time required to disinfect was dependent upon the number of organisms initially present as well as upon the temperature, a fact previously pointed out by Krönig and Paul.[2] These variable factors were controlled as closely

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[1] *Jour. Roy. San. Inst.*, 1903, 24, p. 424.

[2] *Ztschr. f. Hyg.*, 1897, 25, p. 1.

as possible. To these possible variables, which it has been necessary for the present to fix arbitrarily, Chick[1] has added a third; namely, the time element. Instead of noting the time required for disinfection with varying concentrations of the disinfectant, she determines the concentration necessary to disinfect in a given time. She selects 30 minutes arbitrarily. In discussing the time element it is pointed out that in comparing the action of phenol and mercuric chloride upon *B. paratyphosus* at 20° C., the concentrations necessary to kill in 2.5 minutes have a ratio of 13.6 while those necessary to kill in 30 minutes have a ratio of 550. The selection of a constant time factor is therefore necessary if a constant carbolic acid coefficient is to be obtained. A coefficient thus obtained is truly indicative of the relative value of the disinfectant in question only at the concentration at which the test was made. At any other concentration a new coefficient would be obtained.

Chick therefore establishes three arbitrary conditions of experiment for the comparison of disinfectants. The temperature must be standard, preferably 20° C.; the initial number of bacteria must be the same in each comparison; and the time required to complete the disinfection must be fixt at 30 minutes, or some other constant value.

In thus fixing the variables entering the problem it is indeed possible to determine relative values or carbolic acid coefficients, under the restricted conditions of the comparison. But are not these variables precisely the ones which are not fixt in practice? Given the relative values of two disinfectants at 20° C., what relation exists at 0° or at 40°? Mercuric chloride will destroy germs in 30 minutes at a concentration of only two one-thousandths of that required by phenol for the same result. If results are desired in two or three minutes, however, the concentration must be about one-tenth of that of phenol. We may want a comparison under these conditions or under longer periods of time, four or twenty-four hours. In brief, by the establishment of arbitrary fixt conditions in our comparisons we deprive the results of their practical value. Such a course might still be permissible if the work

[1] *Jour. Hyg.*, 1908, 8, p. 92; also Chick and Martin, *ibid.*, p. 654.

involved in determining the complete relationships were very arduous or if the mathematical expression of these relationships were unwieldy or unusable. Such, however, is not the case. Not one constant, but three, one for each variable condition met with, are necessary to completely describe a disinfectant with reference to phenol and to one type of bacterium. This latter is the only variable entering the practical problem which must be arbitrarily fixt in the experiment. It seems at present to be quite necessary to determine the relative germicidal values of two disinfectants upon the actual kind of germ upon which they are to be employed and to qualify the final comparative results accordingly.

With this one fixt factor, however, and by the aid of certain simple principles of physical chemistry, it is possible to derive mathematical expressions involving all the other variables in the complete problem. These expressions are convenient to use and may be put in such form that the actual labor of making the tests is reduced to a minimum. A brief discussion of the principles involved will be necessary to their clear understanding. Unfortunately the calculus must be resorted to, but when possible the actual meaning of the differential expression is stated.

The particular law of physical chemistry which will be considered first is known as the law of velocity of reaction. It may be briefly stated as follows:

In any chemical reaction the amount of change in the reacting substances in unit time is directly proportional to some power of the concentrations of those substances. Just what factors determine the power in question need not be considered at present. It is sufficient to state that, in what are known as monomolecular reactions, it is the first power that is involved so that, in these cases, it may be stated that the velocity of the reaction or the amount of change in unit time is directly proportional to the concentration of the reacting substance.

It has been shown furthermore, by Madsen and Nyman,[1] and more conclusively by Chick, that the killing of bacteria by disinfectants simulates a monomolecular reaction in which the bacteria take the place of one of the reacting substances. The other reagent,

[1] *Ztschr. f. Hyg.*, 1907, 57, p. 388.

the disinfectant, is present in such excess that its concentration is not materially altered during the process. The effect of varying concentrations of the disinfectant will be discussed separately. For the present also the temperature will be considered constant. Under the assumed conditions, if b represent the number of bacteria present in unit volume after any time, t, has elapsed, we have the differential expression,

$$-\frac{db}{dt}=kb$$

where k is known as the velocity constant. This is merely a mathematical expression of the law cited above, and means that the change in the number of bacteria per unit time is proportional to the number present. To determine the total change in any elapsed time this is integrated to

$$\log \frac{B}{b}=kt$$

in which B is the number of bacteria initially present. The test of the applicability of this formula is the constancy of k under constant temperature conditions but with varying values of t, B, and b. The experiments previously cited have shown that k varies with the kind and concentration of the disinfectant, with the temperature, and with the bacterial species, but otherwise it is constant in each experiment. In other words, this constant is a definite measure of the value of the disinfectant. It indicates the rate of disinfection. The larger the value of k the more rapid the disinfection and the more efficient the disinfectant. It may be compared directly with the similar constant obtained with phenol. It will be noted particularly that k is independent of the initial number of bacteria present and furthermore that it does not involve complete disinfection. The point of practically complete disinfection is indefinite and its determination involves large percentage error. With such a formula as that given above, a point of 50 per cent reduction would serve the purpose much better, and a degree of accuracy is secured with a small number of tests which would necessitate many times that number under the other method.

The effect of varying the concentration of disinfectant must next be considered. Returning to the original equation and includ-

ing now the concentration of the disinfectant as well as of the bacteria, we obtain

$$-\frac{db}{dt}=KbC^n$$

in which C is the concentration of the disinfectant expressed in any convenient form, and n, an exponent indicating the order of the reaction. As previously pointed out, in a monomolecular reaction, n is unity, but for the disinfectant, n may have another value and must be considered. This constant, n, expresses mathematically what Chick has pointed out experimentally, that mercuric chloride may have a "carbolic-acid coefficient," of 13 at one concentration and 550 at another. If, therefore, n can be determined, it will be unnecessary to adopt a standard time of testing, and the properties of the disinfectant will be more fully determined. Integrating again

$$\log\frac{B}{b}=KC^nt$$

Hence, KC^n is the k of the previous expression. It varies with the concentration and may be determined as already indicated for two concentrations of disinfectants. Let the values be k and k', C and C' respectively. Then

$$n=\log\frac{k'}{k}\div\log\frac{C'}{C}\text{[1]}$$

Having C and n, then it is possible to calculate K. This is the true velocity constant of the disinfectant, being independent of the concentration, and thus differing from k which is constant only at constant concentrations. The constant, n, may be called the concentration exponent. It defines the relative strengths of two different concentrations of the same disinfectant. The two constants, K and n, therefore describe the fundamental characteristics of a disinfectant at any given temperature. Before discussing them in detail, the relation of temperature may be considered. A second law of physical chemistry has been empirically established as follows:

As the temperature increases in arithmetical progression the

[1] Common logs may be used.

velocity of reaction increases in geometrical progression; or mathematically expressed

$$\frac{K'}{K}=\theta^{(T'-T)}$$

in which K' and K are the constants of the reaction at the temperatures T' and T respectively and θ is the temperature coefficient. It has been found in the case of most chemical reactions that the velocity increases from two to threefold for each rise of 10° C. Among the bacterial reactions, however, the increase is greater—varying, according to Chick, from two to fourfold in the case of metallic salts, to seven or eightfold for phenol and similar compounds. A third constant, the temperature coefficient, is therefore necessary to express this characteristic of a disinfectant. The agreement of the disinfecting reactions with the temperature law of chemical reactions has been satisfactorily demonstrated in many instances and may safely be assumed in all cases. Comparisons made at two temperatures 10° apart furnish a basis for the calculation of the temperature coefficient. With this coefficient determined, the value of the velocity constant at any temperature may be calculated from the following:

$$K_{T^\circ}=K_{20^\circ}\times\theta^{(T-20)}$$

These three constants, the velocity constant, the concentration exponent, and the temperature coefficient, define three essential characteristics of the disinfectant. The last two are independent of experimental conditions. The first, however, is determined also by the type of test organism employed. Therefore any standardization of a disinfectant must be referred to a particular organism. To further correct the result for the uncertain resistance of the particular culture used, the value of K may be divided by the value similarly obtained for phenol. This gives a coefficient independent of all variables except the nature of the test organism. If the temperature were not 20°, reduction can be made by the formula above.

The minimum amount of work required to determine the three constants of a disinfectant is much less than is called for by the present methods. The end point to be used will be a reduction of

from 50 to 75 per cent of the initial number, altho other points serve practically as well if not too near either end. The plate method can therefore be used and the organisms counted. Before adding the disinfectant, the number of bacteria in the initial water suspension is determined. To two tubes, disinfectant in two different concentrations is added. A third tube is treated like one of these and is kept at a temperature approximately 10° higher. After a convenient length of time, depending wholly upon the character of the organism and the strength of the disinfectant, the number of bacteria present in each tube is determined with the usual precautions as to neutralization of disinfectant and other details.

At the same time a single determination of K is made on phenol using the same bacterial culture. The value of n for phenol is found to be 6, and of θ, between 7 and 8. These apparently do not vary with the test organism. From the results from these four tubes, tested initially and finally, the ratio of the velocity constants of phenol and the unknown germicide, and the temperature coefficient and concentration exponent of the latter can be determined. Of course in a careful comparison one or two intermediate determinations of numbers would be made between the initial and final sets. Each additional determination allows of an independent determination of K, the results of which should then be averaged. As a matter of fact the mathematical and theoretical considerations are more accurate than the experimental work. Invariably when a large number of tests are made the average results are in conformity with the theory.

For the purpose of illustration an imaginary case has been worked out using the K, n, and θ values which have been calculated from a large number of experiments by Madsen and Nyman, Chick, and in the writer's laboratory. This illustration is given to show the mode of procedure, the amount of labor involved in the test, and the method of making the calculations.

To summarize, disinfectants differ in three independent ways and there are required three constants to indicate their relative efficiencies. These efficiencies are not determined by a comparison of one of the factors any more than the relative volumes of two pieces of wood are determined by a comparison of their lengths.

TABLE 1.

RESULTS OF TEST: ANTHRAX SPORES PER CUBIC CENTIMETER.

TIME (MINUTES)	PHENOL	MERCURIC CHLORIDE		
	5 per cent	1 per cent	0.5 per cent	
	20°	20°	20°	29°
0	125,000	125,000	125,000	125,000
2	......	......	......	15,500
5	......	7,900	......	630
10	......	545	9,250	4
15	......	27	......	......
20	......	......	720	......
30	......	......	60	......
60	112,000	......	......	......
120	101,000	......	......	......
240	82,000	......	......	......

CALCULATIONS.

Using the formula

$$\log \frac{B}{b} = KC^{n}t$$

expressing time in minutes, concentration in per cent, and using common logs, we obtain the following values of KC^{n}:

TABLE 2.

VALUES OF KC^{n}.

TIME (MINUTES)	PHENOL	MERCURIC CHLORIDE		
	5 per cent	1 per cent		0.5 per cent
	20°	20°	20°	29°
0	......	....	....	....
2	......	....	....	.453
5	......	.240	....	.440
10	......	.236	.113	.460
15	......	.244	....	....
20	......	....	.112	....
30	......	....	.110	....
60	.00080	....	....	....
120	.00078	....	....	....
240	.00077	....	...	....
Average	.00078	240	.112	.451

Having the value of $n=6$ already established for phenol, we obtain for this disinfectant

$$K_{20} = .000,000,05.$$
$$= .05 \times 10^{-6}$$

For the mercuric chloride we have at 20°:

$$KC^{n} = 0.240 \text{ at } C=1$$
$$KC^{n} = 0.112 \text{ `` } C=0.5$$

Whence

$$n = \log \frac{.240}{.112} \div \log \frac{1}{0.5} \ldots\ldots n = 1.08$$

$$K_{20} = 0.24 \text{ at } C = 1 \ldots\ldots K_{20} = 0.24$$
$$0.24 \text{ `` } C = 0.5$$

at 29° and $C = 0.5$

$$KC^n = .451$$

Whence

$$K_{29^\circ} = 0.953$$

$$9 \log \theta = \log \frac{K_{29}}{K_{20}} = 0.600$$

$$\log \theta = 0.067 \ldots\ldots\ldots\ldots \theta = 1.17.$$

Disinfectants vary in the rapidity of their action. Other things being equal, the disinfectant which accomplishes a certain destruction of bacteria in a given time is twice as efficient as one which accomplishes the same result in twice the time. This velocity factor we denote, in keeping with the nomenclature of physical chemistry, by K which we call the velocity constant. It indicates the velocity of the disinfection.

The second point of difference among disinfectants is the change in efficiency which they show with changing concentrations. Thus a 0.2 per cent mercuric chloride solution is twice as efficient (will do the work in half the time) as a 0.1 per cent solution. With phenol, however, in dilute solution, doubling the strength increases the efficiency or decreases the time 64 times. Conversely, halving the strength decreases the efficiency or increases the time 64 times. Other disinfectants have other ratios of increase with increasing strength. This factor we denote by the concentration exponent, n. This constant shows by what power of 2 the efficiency is increased if the concentration be doubled.

The third point of difference among disinfectants is their relation to temperature. Increasing the temperature 1° may increase the rapidity of disinfection by from 1.074 to 1.123 fold, or for 10° the change would be from 2 to 8 fold. The temperature coefficient, θ, shows how many fold the efficiency is increased by a rise of one degree in temperature.

Given these three constants of a disinfectant, results obtained at any one concentration, duration of time, and temperature can

be transformed to any other set of conditions by the following equations:

$$\log \frac{B}{b} = KC^n t$$

$$K_{T^\circ} = K_{20^\circ} \cdot \theta^{(T-20)}$$

B	Initial number of bacteria present.
b	Final " " " "
t	Elapsed time.
C	Concentration of disinfectant.
K	Velocity constant calculated at temperature of the experiment.
K_{T°	Same at any temperature T°.
K_{20°	Same at any temperature 20°.
n	Concentration exponent.
θ	Temperature coefficient.

The point of view which is taken here also somewhat simplifies our conception of antiseptic as distinguished from germicidal properties. It must be admitted that with our ordinary methods of determining antiseptic properties there is no attempt made to determine whether there is or is not a true killing of the organism. The experiment is made with a very low concentration of disinfectant and proceeds for a long time. There is an apparent inhibition under these conditions, which may be really a true killing. It is evident from the form of our killing curve that a low rate of killing over a long time is just as efficient as a higher rate for a shorter period. There is no apparent reason thus far for the distinction between germicidal and antiseptic properties.

The real difficulty appears when two substances are compared in these respects. The fact, now well established, that one of the two may be the superior germicide and the other the superior antiseptic, leads at first thought to the belief that the two properties, germicidal and antiseptic, are unrelated and distinct. A brief consideration of the principles which have been laid down here will show that such a contention is at least unnecessary and probably unjustified.

Two disinfectants having different values of the concentration exponent, n, will have different relative efficiencies at different concentrations. Their efficiency-concentration curves may and in fact often do cross, so that at the point of crossing they are equally

efficient, while above that point the one, and below that point the other, is superior. It follows then that antiseptic action, or true disinfection at low concentrations for long times, will show a relation between two disinfectants quite different from that shown at higher concentrations and shorter times. A disinfectant with a high value of n loses efficiency rapidly with dilution and hence is found to be a poor antiseptic, while one with a smaller value of n loses its power less rapidly with dilution. It has already been shown that decreasing the concentration of phenol one-half decreases its efficiency 64 times, its concentration exponent being six; while mercuric chloride with an exponent of one suffers a loss of efficiency of only one-half by similar dilution.

It is hoped to present this matter more fully in a subsequent paper and to support it with experimental data. For the present it is sufficient to point out that there is no necessity for the distinction between germicidal and antiseptic properties, and that the latter are equally determined by the fundamental constants that are here proposed.

An interesting corollary may also be drawn from this discussion. The velocity constant measures the velocity of the reaction between the disinfectant and the germ. With a given germ it is a measure of the power of the disinfectant. With a given disinfectant it is likewise a measure of the resistance of the organism. Hitherto all attempts to express the relation of bacteria to disinfectants, including heat, have been arbitrary. Death points have been sought when no such points exist. The rate of dying, whether under the influence of heat, cold, or chemical poison, is unfailingly found to follow the logarithmic curve of the velocity law, if the temperature be constant. This curve never reaches a zero value altho approaching it indefinitely. When in practice the bacteria are all killed this fact is interpreted mathematically to mean that, in the volume tested, the number is less than one. The common observation that the larger the initial number the longer the time required to kill is a proof of this view. It requires a greater percentage reduction to reduce the number to less than one, the larger the initial numbers. Therefore these death points are merely accidental, depending in the first place wholly upon the technic adopted

and again upon the chance of the last few survivors living or dying, when for a considerable period of time these chances are about equal.

The velocity constant, K, defines the properties of the logarithmic curve, and hence can be made to define such fundamental properties of an organism as its resistance to heat. To measure this function we would not attempt to determine a temperature at which the organisms all die (the non-existent thermal death point) but rather the rate of dying at any fixt temperature. If then we add to this determination a determination of θ which shows how this rate of dying increases with rising temperature, we have fully defined the thermal properties of the organism. A similar line of reasoning would apply to the relation of the organism to any other germicidal agent.